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EFFECT OF ISCHAEMIA-REPERFUSION ON GLUTATHIONE PEROXIDASE, GLUTATHIONE REDUCTASE AND GLUTATHIONE TRANSFERASE ACTIVITIES IN HUMAN HEART PROTECTED BY HYPOTHERMIC CARDIOPLEGIA

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The activities of glutathione peroxidase (GSH-px), glutathione reductase (GSSG-rx) and glutathione transferase (GST) were measured in myocardial specimens obtained from right atria of patients subjected to different period of ischaemic arrest (aortic clamping ranging from 10 min to 90 min) followed by 60 min. of reperfusion, during open heart surgery 41–90 min. period of aortic clamping induced a significant increase of GSH-px activity with both H_2O_2 ($p < 0.05$) and cumene hydroperoxide ($p < 0.025$) as substrates when compared with baseline levels. Aortic clamping and reperfusion, however did not significantly change the myocardial activities of glutathione transferase and glutathione reductase. It is suggested that the increase of GSH-px in ischaemic-reperfused human hearts may render the myocardium less susceptible to oxidative attack particularly during the reoxygenation period when the level of active oxygen species is greatly elevated.

KEY WORDS: Ischemia-reperfusion, cardioplegia, human heart, glutathione peroxidase, glutathione transferase, glutathione reductase.

INTRODUCTION

It has been suggested that active oxygen species, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\cdot$), are implicated in ischaemia-reperfusion myocardial injury[1–5] through the peroxidation of structurally important polyunsaturated fatty acids within the phospholipid structure of the membranes (lipid peroxidation). H_2O_2, in particular, could play a major role in the pathogenesis of free radical-mediated tissue damage, primarily because of its ability to react with superoxide anion (O_2^-) in the presence of metal ions to form the highly cytotoxic hydroxyl radical ($OH\cdot$).[6]

Among the enzymatic systems capable of protecting cells against oxidative injury,

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glutathione peroxidase (GSH-px), glutathione reductase (GSSG-rx) and glutathione transferase (GST) play a crucial role. Using GSH as cofactor, the selenium dependent glutathione peroxidase (Se-GSH-px) reduces H_2O_2 to water and organic hydroperoxides to the corresponding alcohols.[7] This reaction leads to the conversion of GSH into its oxidized form (GSSG). In the presence of NADPH, glutathione reductase is able to reduce the oxidized glutathione. Glutathione transferase is a multifunctional enzymatic system which, besides conjugating glutathione to a large number of xenobiotics, also has the capacity of catalyzing the GSH peroxidase reaction with organic hydroperoxides (non-Se-GSH-px) as substrates.[8] Furthermore, as GST accepts 4-hydroxyalkenals as a substrate, it is therefore capable of detoxifying these biologically reactive products arising from oxidative metabolism.[8] Interestingly, in the rat heart, the prominent glutathione transferase isoenzyme GST 4–4 shows a good activity with 4-hydroxynonenal.[10] The response of the antioxidant defense system during ischaemia-reperfusion has been extensively studied using hearts from different animal species.[1,11–13] Although there is a growing interest in understanding the biochemical events occurring during ischaemia-reperfusion in man, no data on the activities of these protective enzymes in the human heart are reported in the literature.

During open heart surgery with cardio-pulmonary bypass, a transient global ischaemia is produced which represents a suitable model for studying the effects of ischaemia-reperfusion in humans. The present study was carried out in an attempt to measure the baseline levels of GSH-px, GSSG-rx and GST activities in human heart and to investigate if cardioplegic arrest followed by reperfusion (open heart surgery) can induce significant changes in myocardial activities of the above mentioned glutathione-dependent antioxidant enzymes.

MATERIALS AND METHODS

Patients

Forty three patients (30 men and 13 women, mean age 54 years) undergoing open heart surgery, 10 for valve replacement and 33 for elective cornary revascularization, were included in the study. All the patients were premedicated with promethazine (0.5–1 mg/Kg, i.m.), atropine (0.01 mg/kg, i.m.) and petidine (1 mg/kg, i.m.) 1 hour before sugery. Patients were anaesthetized with fentanyl (0.005 mg/kg), sodic thiopentone (3–5 mg/kg) and were ventilated wih 100% oxygen during the operation. Monitoring included a radial arterial line, an ECG and nasopharyngeal temperature.

Surgical Technique

Cardiopulmonary bypass with a bubble oxygenator and a roller pump was instituted with atrial or bicaval and aortic arch cannulation. Moderate systemic (26–28°C) and local hypothermia (topical ice cooling), aortic occlusion (aortic clamping) with the injection of a hyperkalaemic crystalloid cardioplegia solution (St. Thomas solution)[14] were employed to protect the heart during the operation (aortic occlusion time ranged 10–90 minutes). Cardioplegic solution was given every 20 minutes during aortic occlusion to maintain myocardial temperature below 15°C. At the end of the surgical operation the aortic occlusion was removed and the heart reperfused with oxygenated blood.

Sample Collection

Specimens (100–500 mg) of the right atrial myocardium were removed at the beginning of the operation (before aortic clamping) and 60 min after aortic clamp removal. During this 60 min. period hearts were reperfused with oxygenated blood. The samples were immediately transferred to isotonic saline solution, washed thoroughly and stored at −70°C until used. No loss of enzymatic activity was noted after storage for at least two months. According to the clamping time (duration of ischaemic period) the samples investigated were divided into two groups. In the first group (20 patients) the hearts were subjected to 10–40 minutes of global ischaemia, whereas in the second group (23 patients) the hearts were subjected to 41–90 minutes of global ischaemia.

Supernatant Preparation

Each sample was weighed, minced and homogenized with two 20-sec bursts of an Ultraturrax homogenizer, allowing 30-sec rests between bursts in 4 vol of 10 mM phosphate buffer, pH 7, containing 1 mM EDTA and 1 mM dithiothreitol. The homogenate was centrifuged at 50,000 × g for 60 min. and the resultant supernatant immediately used for determination of enzymatic activities.

Assays

GSH-px activities were measured according to the method of Paglia and Valentine.[15] The final concentration of peroxides was 0.25 mM for H_2O_2 and 1.2 mM for cumene hydroperoxide (CHP). The assay solution contained 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 1.5 mM NaN_3, 1 mM GSH, 0.16 mM NADPH, 4 μg of glutathione reductase and a suitable sample (0.4–0.8 mg) of enzyme solution. After 5 min. of preincubation the reaction was started with the addition of peroxide. The value for a blank reaction with the enzyme source replaced by water was subtracted for each assay. The rate of reaction was recorded at 25°C by following the decrease in absorbance at 340 nm. Specific activity was expressed as nanomoles of GSH oxidized per minute per mg of protein.

GST activity was measured by method of Habig *et al.*[16] The assay mixture contained 0.1 M potassium phosphate buffer pH 6.5, 1 mM EDTA, 2 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene and a suitable amount (0.04–0.08 mg) of enzyme source. The reference cuvette contained the complete assay mixture with the enzyme replaced by water. Enzymatic assay was carried out at 25°C. Specific activity was expressed as nanomoles of GSH conjugated per minute per mg of protein. GSSG-rx activity was determined as described previously.[17] The assay mixture contained 0.1 M potassium phosphate buffer pH 7.4, 1 mM EDTA, 1 mM GSSG, 0.16 mM NADPH and an appropriate amount (0.4–0.8 mg) of enzyme source. The blank did not contain GSSG. Enzyme activity was determined at 25°C by measuring the disappearance of NADPH at 340 nm and expressed as nanomoles of NADPH oxidized per minute per mg of protein. All measurements, in a final volume of 1 ml, were performed in duplicate and at least at two different protein concentrations.

The method of Bradford[18] was employed for the determinations of protein concentrations. Gamma-globulin was used as protein standard.

Statistics

The results are expressed as mean ± S.E.M. To determine significance paired and unpaired Student's *t* tests were used when appropriate. Values of $p < 0.05$ were considered significant.

RESULTS

The data are reported in Table 1 and Figure 1.

Our data show that when the duration of the cardioplegic arrest ranged between 41–90 min. there was a significant increase in GSH-px myocardial activity as compared to basal values (H_2O_2-GSH-px from 21.2 ± 0.83 to 24 ± 1.25, $p < 0.05$; CHP-GSH-px from 20 ± 0.81 to 23.4 ± 1.43, $p < 0.025$). On the contrary, when the clamping time was 40 min or less, the myocardial GSH-px activity was unchanged with respect to baseline. The myocardial activities of GSSG-rx and GST did not show any significant change in both groups.

DISCUSSION

The present study shows that prolonged ischaemia (41–90 min.) and subsequent blood-reperfusion (60 min.) significantly increases the activity of GSH-px in the human heart. In order to determine the fractions of the selenium-dependent and selenium-independent GSH-px, the enzymatic activities were recorded using both H_2O_2 and CHP as substrates. The results indicate that the activities measured with H_2O_2 were as high as those obtained with CHP. This data shows that GSH-px is largely associated with the selenium-dependent form in the human heart. Consequently, the increase in GSH-px activity must be attributed to the selenium-dependent enzyme. Furthermore such an increase seems to suggest that oxygen radical production is not completely suppressed by the hypothermic cardioplegic solution, usually employed to protect the myocardium against the ischemic damage during open heart surgery.

Since GSH-px is highly effective in the inactivation of H_2O_2 and organic by hy-

TABLE 1

Effect of aortic clamping time (duration of cardioplegic arrest) on myocardial glutathione-dependent enzymes

Enzyme	Base	A.C. ⩽ 40	A.C. ⩾ 40
GSH px			
H_2O_2	21.2 ± 0.83	22.0 ± 1.22	24.0 ± 1.25*
CHP	20.0 ± 0.81	21.3 ± 1.33	23.4 ± 1.43**
GST	45.7 ± 3.41	46.0 ± 3.78	52.7 ± 6.02
GSSG rx	17.1 ± 1.14	16.4 ± 1.20	16.4 ± 1.52

GSH-px activity is expressed as nmol of GSH oxidized per min. per mg.

GST is expressed as nmol of GSH conjugated per min. per mg.

GSSG-rx is expressed as nmol of NADPH oxidized per min. per mg.

A.C. ⩽ 40 = aortic clamping time between 10–40 min.; A.C. ⩾ 40 = aortic clamping time between 41–90 min.

The data are presented as means ± S.E.M. The means ± S.E.M. were calculated from the data reported in Figure 1.

* = $p < 0.05$, ** = $p < 0.025$

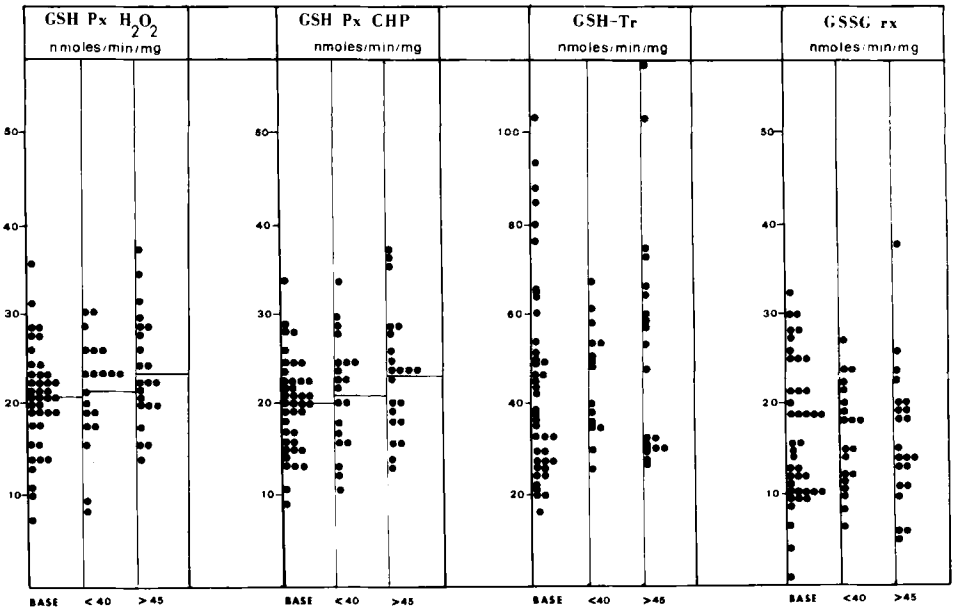

FIGURE 1 Glutathione peroxidase, glutathione transferase and glutathione reductase activities in human heart samples obtained before and after aortic clamping.
A.C. $\leqslant$ 40 = aortic clamping time between 10–40 min.; A.C. $\geqslant$ 40 = aortic clamping time between 41–90 min.

droperoxides, an increase in its activity may reduce the heart susceptibility to oxidative attack, particularly during the reoxygenation , which greatly increases the production of active oxygen species. Accordingly, Menasche *et al.*[19] recently demonstrated that the addition of hypoxic cardioplegic solution with peroxidase, rather than superoxide dismutase and catalase, significantly enhances the protection of the globally ischaemic, reperfused rat heart, as shown by a better recovery of contractile indexes. The increase in GSH-px in the ischaemic reperfused heart may be crucial, considering that this enzyme has been also found more effective in the protection of cells exposed to O_2 than catalase and superoxide dismutase.[20] Our results seem to agree with a recent study, showing GSH-px activity with H_2O_2 as substrate was significantly increased in the rat heart subjected to 60 min. ischaemia followed by 30 min. reperfusion.[11] In contrast, Juliker *et al.*[12] found that GSH-px of the rat heart was not affected by ischaemia (60 min.) followed by reperfusion. On the other hand, after coronary ligation in rats[2] and dogs[1] or after prolonged hypoxic period, a decrease in GSH-px has been reported in crude preparations.[21] Ferrari *et al.*[22] found that GSH-px activity was not affected throughout the experiment in an ischaemia-reperfusion model of the isolated rabbit heart. Finally, Shlafer *et al.*[23] found that two hours normothermic global ischaemia followed by one hour reperfusion slightly decreased rabbit heart GSH-px activity. The results presented in the literature are therefore controversial. The reasons for such disparity may be due to differences in ischaemia protocols and assay methods, as well as in animal species. It is noteworthy to outline that all the above mentioned studies were carried out in normothermic conditions and using experimental models. On the contrary, our data were obtained

on human hearts protected during the ischaemic period by hypothermic cardioplegia. Therefore it is intriguring to compare our results with those reported in the literature.

Whether or not de novo synthesis of the enzyme, possibly in response to increased H_2O_2 formation, is responsible for enhanced GSH-px activity is difficult to state. However, an induction in GSH-px activity stimulated by metabolic H_2O_2 production *in vivo* has recently been reported.[24] On the other hand, several possibilities, such as activation resulting from partial proteolysis or removal of enzymatic inhibitors or other unknown mechanisms, could explain our observations. Our data also demonstrated that GSH-px is stable in the face of ischaemic injury. This data agrees well with the report of Condell and Tappel, [25] showing that GSH-px activity is very stable when subjected to oxidative stress, proteolysis and denaturation. In conclusion, the increase in GSH-px activity, may provide an effective defence of the human heart against H_2O_2, organic hydroperoxide and the products of lipid peroxidation generated during ischaemia-reperfusion.

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